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CYTOLOGICAL STUDIES OF OEDOGONIUM. II. CHROMOSOME NUMBERS IN TWELVE SPECIES OF OEDOGONIUM[1]

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ABSTRACT

Mitotic chromosome counts are reported for 12 species of *Oedogonium* with the following distribution: two species with 13 chromosomes, two species with 16 chromosomes, five species with 17 chromosomes one species with 19 chromosomes, one species with 32 chromosomes, and one species with 38 + 1 chromosomes. Diploid strains of two species are illustrated and discussed. Cytological comparison of species establishes that there is great diversity in *Oedogonium* with relation to chromosome number, size, and morphology.

THE NUMBER of validly descr'bed species of *Oedogonium* presently exceeds 475. From this large group chromosome numbers have been determined for only 11 identified species. This, of course, represents insufficient information to determine clearly the limits of cytological diversity that might exist among this large number of species and is totally inadequate to establish group affinities based on chromosome number and morphology. The present investigation was undertaken as a contribution toward the eventual goal of determining the cytological trends within the genus *Oedogonium*.

The earliest chromosome count in *Oedogonium* was obtained by van Wisselingh (1908) for *Oe. cyathigerum* ($n=19$). Since this early work, counts have been determined for another 10 identified species as well as for 16 unidentified species (Table 2). This paper reports on the cytology of 11 additiona' species and discusses a twelfth species that had been previously studied.

MATERIALS AND METHODS—The 12 species of *Oedogonium* investigated were obtained from the sources indicated in Table 1. Eleven of the species have been identified, but the twelfth species (described in this paper as strain 13) could not be identified. This unidentified species was reproductive when collected and was gynandrosporous. However, sexual material was not preserved at the time of collection, and isolates of this alga failed to reproduce sexually in the laboratory. With the lack of sexual material, species identification was not possible. Nevertheless, since the chromosome condition of this a ga is of interest, a discussion of this unidentified species has been included in this paper.

All 12 species were grown in unialgal culture in autoclaved soil-water medium. The cultures were maintained on a 12:12-hr, light-dark cycle with a light intensity of ca. 300 ft-c and a temperature of ca. 21 C. Illumination was provided by 40-w cool-white fluorescent lamps.

Cytological study indicated that mitosis in all 12 species was rhythmic and influenced by the light-dark cycle as reported for *Oedogonium* by Kretschmer (1930), Bühnemann (1955), and Henningsen(1963). The mitotic rhythm in *Oedogonium* is reportedly controlled by a light-receptor system, most sensitive to light under a wave length of 550 mμ (Bühnemann, 1955). When the cultures were maintained under the conditions described, the maximum number of cell divisions in most species occurred 5–9 hr after the initiation of the dark phase. Newly inoculated cultures were permitted to adjust to their environment for about a week prior to harvesting the material for mitotic study. To determine the peak of mitotic division, acetocarmine preparations (without prior fixation) were made of the material at regular intervals. The relative yield of dividing cells in any given culture was found to vary from day to day. Similar variation was reported for various euglenoids by Leedale (1959) who found that weeks with divisions occurring every night were interspersed with occasional days when no divisions occurred.

Mitotic division in each of the species of *Oedogonium* studied occurred at a predictable time after the onset of darkness. For example, under the conditions described mitotic divisions in *Oe. cardiacum* cultures always began 6–8 hr after the initiation of darkness, irrespective of the time of day. The observation that mitosis in individual *Oedogonium* species occurs at different times after the initiation of the dark period was likewise noted by Henningsen (1963).

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TABLE 1. *Unialgal cultures of Oedogonium investigated*[a, b]

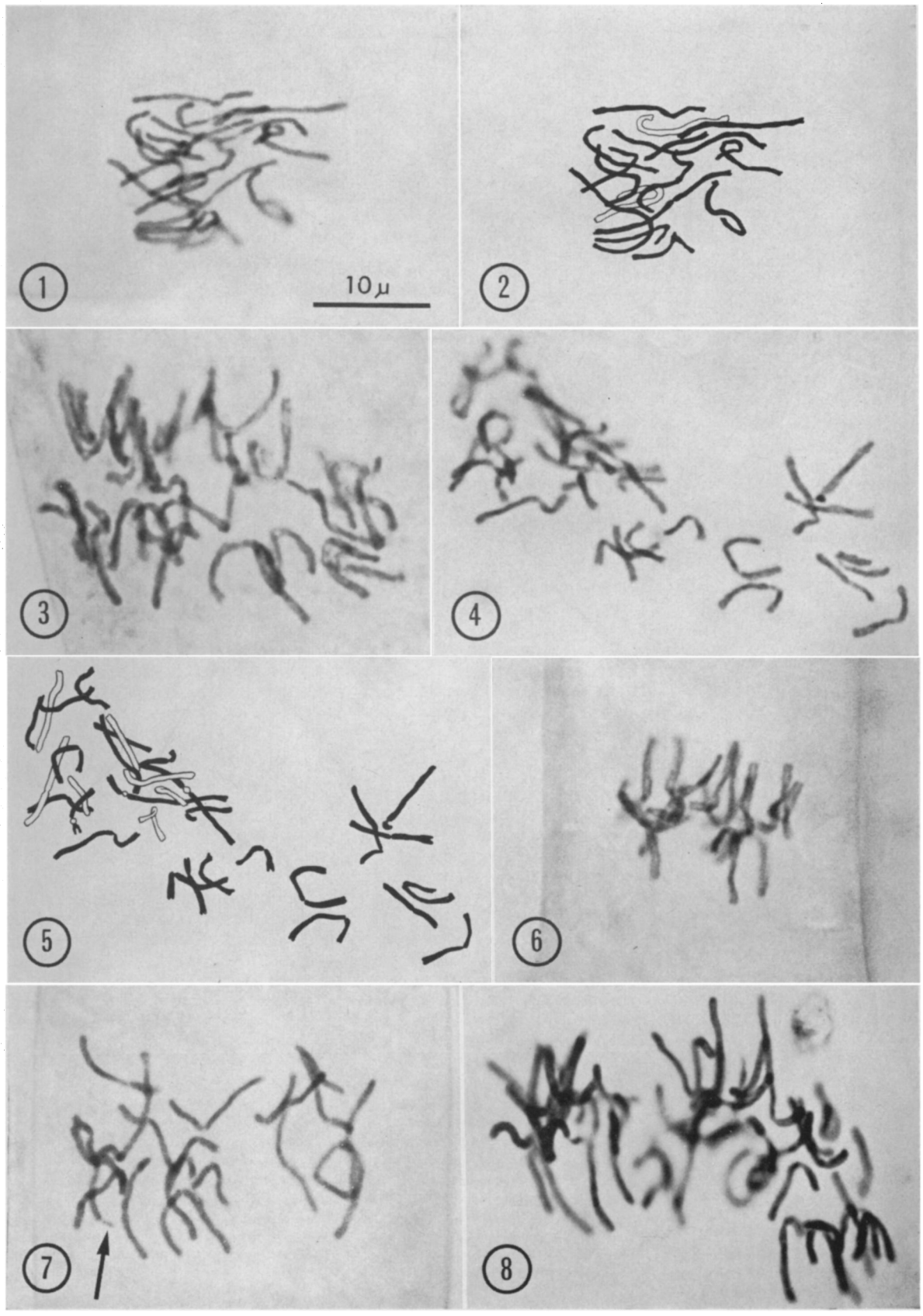
Species	Source	Isolator
1. Macrandrous with bisexual filaments:		
Oedogonium foveolatum Wittr.	Indiana Culture Collection LB933 (Starr, 1964)	Bold
Oe. foveolatum, diploid strain	Derived from the typical haploid strain listed above (Hoffman, 1965)	Hoffman
Oe. geniculatum Hirn	Indiana Culture Collection LB828	Stein
2. Macrandrous with unisexual filaments:		
Oe. angustistomum Hoffman[c]	Radnor Lake, Nashville, Tenn.; male and female strains	Hoffman
Oe. calliandrum Hoffman[c]	Acuff, Texas; male and female strains	Hoffman
Oe. capilliforme Kuetz.; Wittr.	Lake Buchanan region, Texas; male and female strains	Hoffman
Oe. cardiacum (Hass.) Wittr.	Indiana Culture Collection LB40, female; LB39, male	Christensen
Oe. cardiacum, diploid, female strain	Indiana Culture Collection LB847; derived from LB40	Starr
Oe. princeps (Hass.) Wittr.	Jollyville, Texas	Hoffman
3. Gynandrosporous:		
Oe. ringens Hoffman[c]	Llano, Texas	Bold
Oe. setigerum Hoffman[c]	Indiana Culture Collection LB849	Stein
Oedogonium sp.	Dr. R. C. Starr, Germany; designated in this paper as strain 13	Hoffman
4. Idioandrosporous:		
Oe. echinospermum Al. Br.	Austin, Texas; oogonial and androsporangial strains	Hoffman
Oe. idioandrosporum (Nordst. & Wittr.) Tiff.	Two sets of isolates, one from Hillsboro, Texas, another from Lake Buchanan region, Texas; oogonial and androsporangial strains from each locality	Hoffman
5. Sexual condition not known:		
Oedogonium sp.	Lake Buchanan region, Texas; designated in this paper as strain 15	Hoffman

[a] The author is indebted to the late Professor L. H. Tiffany for confirmation of the identifications of some of these species
[b] Voucher specimens of mature sexual material of each species have been deposited in the University of Texas Herbarium. The cultures and permanent slides of the cytological preparations are in the possession of the author.
[c] Recently described species (Hoffman, in press).

When *Oedogonium* cultures were maintained under continuous illumination, mitotic divisions occurred irregularly with no observable rhythm. Young germling filaments, when placed under continuous illumination with air containing 0.5–1% CO_2 bubbled through the medium, underwent division at the rate of two or more divisions per cell per day. This is in general agreement with the observations of Howard and Horsley (1960). In contrast, the rate of cell division of similar material maintained on a 12:12-hr, light-dark cycle was less than one division per cell per day, although division was not strictly rhythmic in the very young germling material as it was in the case of the older filaments.

Algal filaments with dividing cells were fixed in a fresh mixture of absolute ethanol and glacial acetic acid (3:1 v/v) for 1–24 hr. If the material could not be stained the same day it was fixed, it was placed in freshly prepared fixative and stored

Fig. 1–8. Mitotic chromosomes of *Oedogonium*, all × 2000.—Fig. 1. Squash preparation illustrating the 16 metaphase chromosomes of *Oe. foveolatum*.—Fig. 2. Drawing based on photographs and the original preparation illustrated in Fig. 1.—Fig. 3. Metaphase chromosomes of a diploid strain of *Oe. foveolatum*.—Fig. 4. Squash preparation illustrating the 32 metaphase chromosomes of *Oe. geniculatum*.—Fig. 5. Drawing based on photographs and the original preparation illustrated in Fig. 4.—Fig. 6. Squash preparation illustrating the 17 metaphase chromosomes of a male strain of *Oe. capilliforme*. Not all are distinguishable at this one level of focus.—Fig. 7. Squash preparation of the 19 metaphase chromosomes of a haploid, female strain of *Oe. cardiacum*. Note satellite (arrow).—Fig. 8. Squash preparation of the metaphase chromosomes of a diploid, female strain of *Oe. cardiacum*.



in a freezer for use within the next few days. Following hydrolysis in 1 N HCl at 60 C for 17–22 min, the material was first stained with the Feulgen reaction and then subsequently treated with aceto-carmine to intensify staining (Hoffman, 1961). Squash preparations were made permanent according to the technique of Conger and Fairchild (1953).

CHROMOSOME NUMBERS—A summary of the available information relating to chromosome numbers in the genus *Oedogonium* is presented in Table 2. Of the 12 species investigated by the author, two were macrandrous with bisexual filaments, five were macrandrous with unisexual filaments, three were gynandrosporus, and two were idioandrosporous. The two macrandrous species with bisexual filaments were *Oe. foveolatum* with 16 chromosomes (Fig. 1, 2) and *Oe. geniculatum* with 32 chromosomes (Fig. 4, 5). The macrandrous species with unisexual filaments were *Oe. capilliforme* with 17 chromosomes (Fig. 6); *Oe. cardiacum* with 19 chromosomes (Fig. 7, 9), in agreement with previous reports indicated in Table 2; *Oe. princeps* with 17 chromosomes (Fig. 10–13); *Oe. calliandrum* with 17 chromosomes (Fig. 14–16); and *Oe. angustistomum* with 17 chromosomes (Fig. 18, 19). The three gynandrosporous species were *Oe. setigerum* with 16 chromosomes (Fig. 17), *Oe. ringens* with 17 chromosomes (Fig. 20, 21), and the unidentified species (strain 13) with 38 ± 1 chromosomes (Fig. 22–24). The idioandrosporous species were *Oe. echinospermum* with 13 chromosomes (Fig. 25) and *Oe. idioandrosporum* with 13 chromosomes (Fig. 26–28).

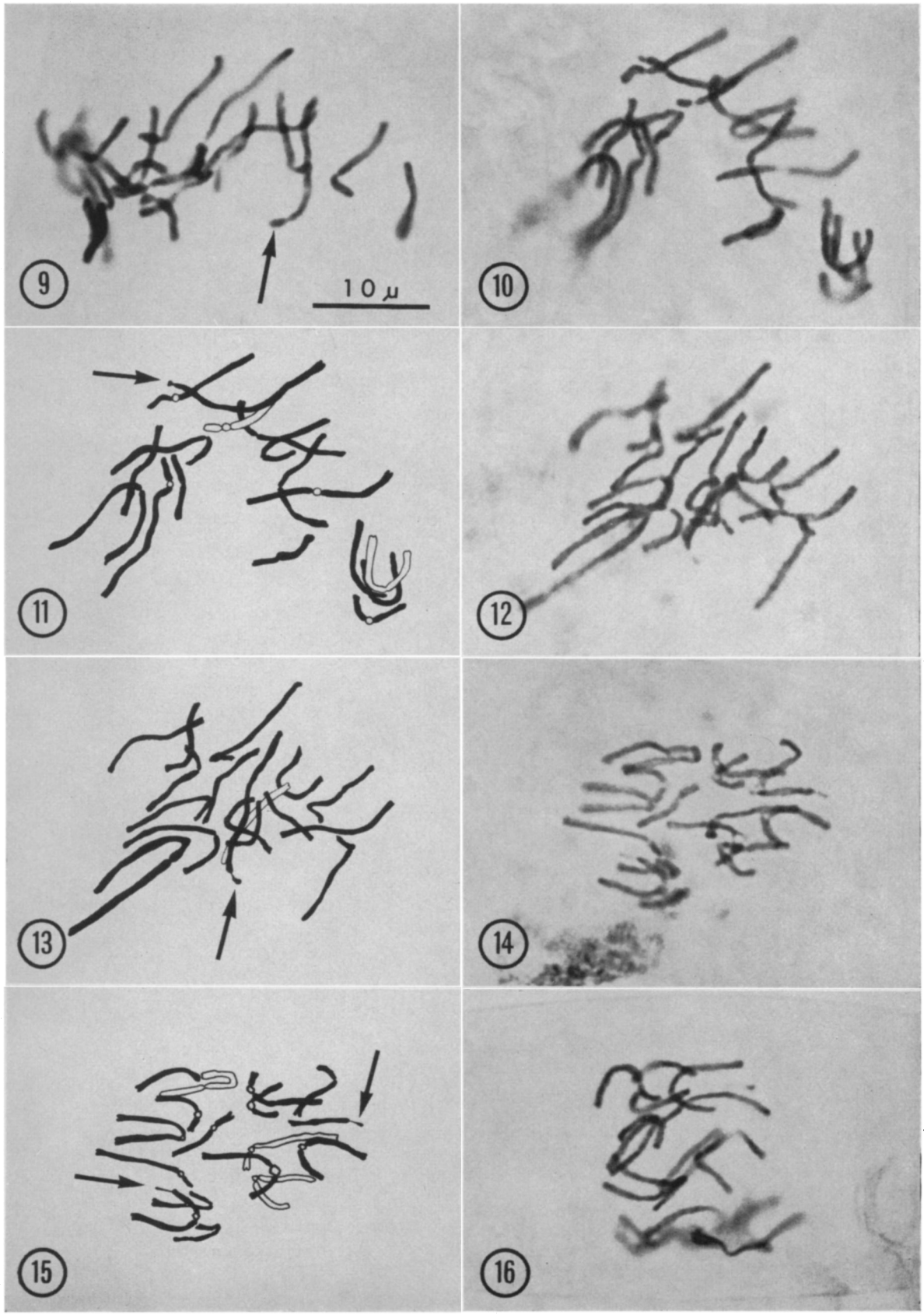
Diploid strains have been obtained and examined cytologically for two of the 12 species investigated. These diploid strains appear to be quite stable in culture; several such strains have been maintained for more than 5 years without reverting to typical haploid condition. In *Oe. foveolatum* diploid strains have been obtained as a result of the failure of meiosis to occur during atypical oospore germination (Hoffman, 1965). The resulting filaments have cells with the diploid chromosome number of 32 (Fig. 3). Diploid strains of *Oedogonium* may also occur spontaneously in culture by somatic doubling of chromosome number, and they can readily be distinguished from their haploid counterparts by their relatively larger cell diameter. This occurs with greatest frequency in old cultures. A diploid oogonial strain of *Oe. cardiacum* was isolated by Starr (Indiana Culture collection of Algae, LB847) from a haploid culture (Fig. 8). Similarly, the author has isolated and examined cytologically diploid strains which arose in old cultures of the male, haploid strain of this species. Other cases of apparent somatic doubling of chromosome number have been observed by the author in cultures of several other species of *Oedogonium*, but these were not confirmed by cytological study.

CHROMOSOME SIZE AND MORPHOLOGY—One of the most striking chromosomal features in *Oedogonium* is the tremendous range of chromosome size found among the various species. The author's observations agree with the generalization reported by Tschermak (1943a) that the total chromosome volume of large-celled species is relatively greater than that of smaller-celled species. This usually means that the large-celled species, in addition to having more chromosomes, typically have larger chromosomes than the smaller-celled species.

Among the author's cultures considerable variation in chromosome size may be seen in the comparison of a small-celled species (strain 15; Fig. 30) and a larger-celled species (strain 13; Fig. 29). The entire metaphase group of chromosomes in the former clearly occupies less volume than a single metaphase chromosome of the larger species. Although chromosome lengths of the two species cannot be measured with absolute accuracy in the preparations studied, it can be determined that the chromosomes of the small-celled species (Fig. 30) are less than 0.5 μ long, while the longest chromosomes in the other species (Fig. 29) are over 20 μ in length at early metaphase. A conservative estimate of relative chromosome size between the two species would be in the order of 1 to 50. This size difference exceeds any previously reported for *Oedogonium* in the literature (Tschermak, 1943a). Since the two species discussed do not represent by any means the smallest or largest species known, even greater contrast in chromosome size probably occurs in the genus.

In most of the species examined the range of chromosome size for a single species was between

Fig. 9–16. Mitotic chromosomes of *Oedogonium*, all × 2000.—Fig. 9. Squash preparation of the 19 metaphase chromosomes of a haploid, male strain of *Oe. cardiacum*. Note satellite (arrow).—Fig. 10. Squash preparation of the 17 metaphase chromosomes of a female strain of *Oe. princeps*.—Fig. 11. Drawing based on photographs and the original preparation illustrated in Fig. 10. Note satellite chromosome (arrow).—Fig. 12. Squash preparation of the 17 metaphase chromosomes of a male strain of *Oe. princeps*.—Fig. 13. Drawing based on photographs and the original preparation illustrated in Fig. 12. Compare the satellite chromosome (arrow) with the homologous chromosome illustrated in Fig. 10, 11.—Fig. 14. Squash preparation illustrating the 17 chromosomes of a female strain of *Oe. calliandrum*. Note chromatids beginning to separate in the region of the centromere.—Fig. 15. Drawing based on photographs and the original preparation illustrated in Fig. 14. Note satellite chromosome (arrow, right) and second chromosome with faintly staining end (arrow, left) which may represent another satellite.—Fig. 16. Squash preparation of the 17 metaphase chromosomes of a male strain of *Oe. calliandrum*.



5–15μ at metaphase. Differences in chromosome length were readily apparent in all species studied, and individual, "homologous" chromosomes could frequently be recognized in different cells of the same species. As reported by Tschermak (1943a) the longest chromosome of a particular species was often quite distinct because of its length.

Other morphological features, in addition to size, which are useful in characterizing individual chromosomes of *Oedogonium* are the position of centromeres and the presence or absence of satellites.

Kretschmer (1930) noted in anaphase chromosomes of *Oe pachyandrium* the region in which the chromosomes bent (i.e., the presumed centromere region) but was unable to determine a morphologically distinct centromere. Tschermak (1943a) was apparently the first to describe distinct centromeres for chromosomes of *Oedogonium*.

In the author's material centromeres were frequently quite distinct They appeared in metaphase chromosomes as a constriction or a narrow, unstained (with Feulgen and/or acetocarmine staining) band (Fig. 27). At the initiation of anaphase, as noted by both Kretschmer (1930) and Tschermak (1943a), the chromatids first begin to separate in the region of the centromere, resulting in the formation of a circular configuration (Fig. 14, 26). On the other hand, Tschermak (1943a) found that the chromatids of colchicine-treated chromosomes separated last at the centromeres.

Individual chromosomes may be readily distinguished on the basis of the position of the centromere and the relative lengths of the two "arms." In some cases, however, the position of a centromere could not clearly be determined. Furthermore, the long metaphase chromosomes were often bent and sometimes failed to lie flat enough for precise measurement of chromosome "arm" lengths. It is apparent that idiograms of *Oedogonium* chromosomes may be more accurately prepared on the basis of colchicine-treated material, as evidenced by the work of Tschermak (1943a), Hasitschka-Jenschke (1960), and Henningsen (1963). C-metaphase chromosomes are relatively much shorter than in untreated material, and the position of the centromeres consistently appears more prominent, especially if the chromatids have begun to separate as is often the case.

Satellite-bearing chromosomes have previously been reported for *Oedogonium*.[2] Of the 10 species illustrated by Tschermak (1943a), three apparently had SAT-chromosomes, two species with one each and a third species with two. Hasitschka-Jenschke (1960) reported three pairs of SAT-chromosomes in diploid material of colchicine-treated *Oe. cardiacum*. More recently, Henningsen (1963) found SAT-chromosomes in three of five species studied, two species with two each and a third species with only one.

Five of the 12 species utilized in this study have SAT-chromosomes which are clearly seen in actively growing material not treated with colchicine. In untreated material of *Oe. cardiacum* there is a suggestion of at least two SAT-chromosomes, one of which is very prominent (Fig. 7, 9). Three species, *Oe. princeps* (Fig. 10–13); *Oe. calliandrum* (Fig. 14, 15); and *Oe. angustistomum* (Fig. 18, 19), have one chromosome with a distinct satellite and, in addition, each has a second chromosome with a faintly staining end which might represent anaother SAT-chromosome (cf. Tschermak, 1943a, p. 497). If so, this second SAT-chromosome might be more clearly seen following colchicine treatment. The unidentified species, strain 13 (Fig. 24), had two very distinct SAT-chromosomes.

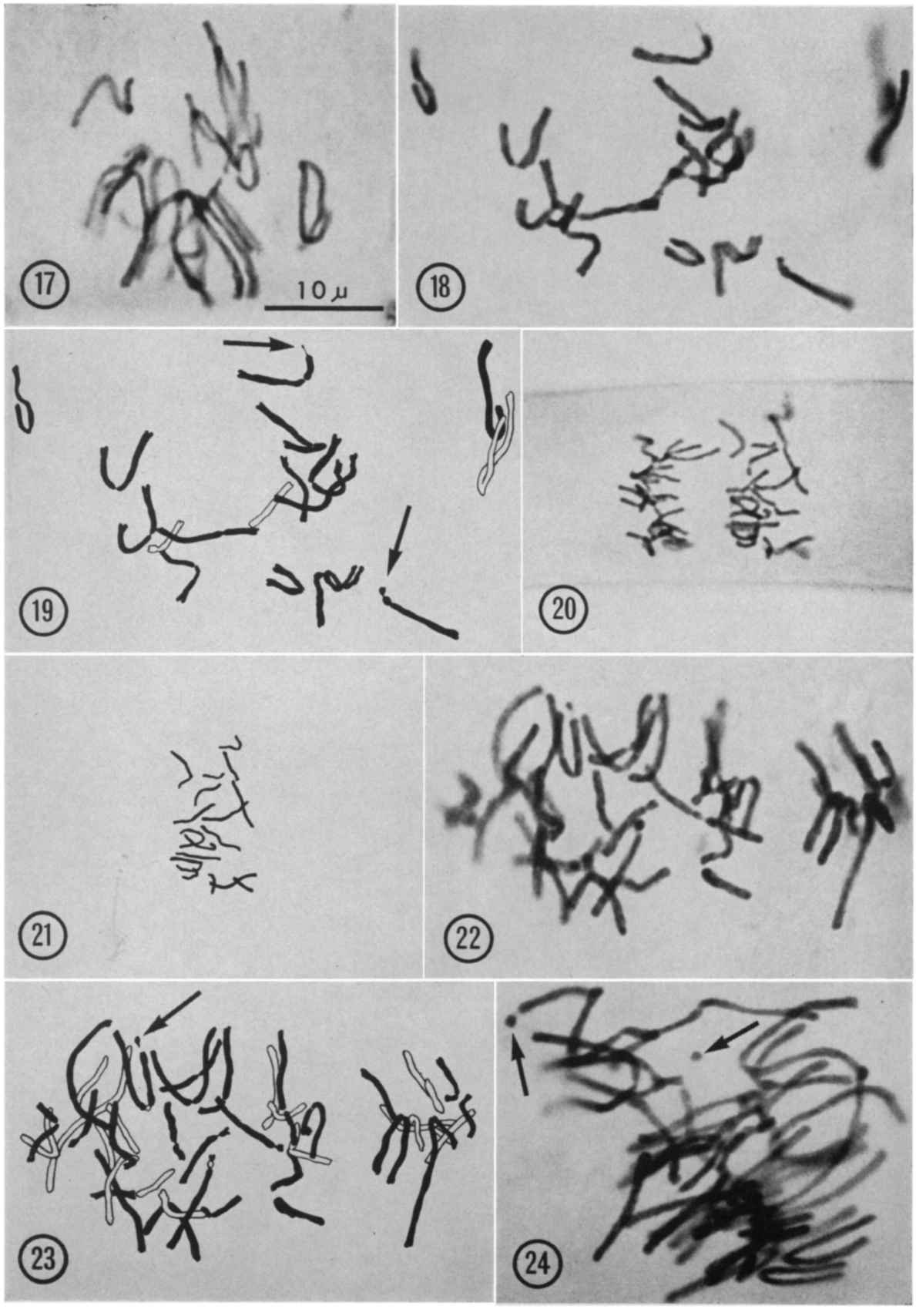
Although the author's material failed to show three distinct SAT-chromosomes for *Oe. cardiacum* as reported by Hasitschka-Jenschke (1960), this might be explained by the differences in technique. It is quite possible that some satellites are much more prominent as seen in C-mitosis. Sinha(1963) did not mention or illustrate SAT-chromosomes for *Oe. cardiacum*.

Henningsen (1963) described two different types of SAT-chromosomes for *Oedogonium*. Such a distinction was not apparent in any of the author's material. Again, direct comparisons could not be made since Henningsen's work was based on C-metaphase chromosomes.

Of the five heterothallic, macrandrous species examined in the present study, the chromosome number and morphology of the complementary male and female strains were indistinguishable.

[2] For a discussion of the possible relationship of SAT-chromosomes and nucleoli in *Oedogonium*, see Tschermak (1943a)

Fig. 17–24. Mitotic chromosomes of *Oedogonium*, all × 2000.—Fig. 17. Squash preparation illustrating the 16 metaphase chromosomes of *Oe. setigerum*.—Fig. 18. Squash preparation of the 17 metaphase chromosomes of a male strain of *Oe. angustistomum*.—Fig. 19. Drawing based on photographs and the original preparation illustrated in Fig. 18. Note satellite chromosome (arrow, right) and second chromosome with a faintly staining end (arrow, left).—Fig. 20. Squash preparation illustrating 2 sets of 17 anaphase chromosomes of *Oe. ringens*.—Fig. 21. Drawing of one group of 17 anaphase chromosomes based on photographs and the original preparation illustrated in Fig. 20.—Fig. 22. Squash preparation of the 38 ± 1 metaphase chromosomes of an unidentified gynandrosporous species (strain 13).—Fig. 23. Drawing based on photographs and the original preparation illustrated in Fig. 22. Only one satellite chromosome (arrow) was distinguishable in this preparation.—Fig. 24. Squash preparation of the same material (strain 13) illustrating two satellites (arrows).



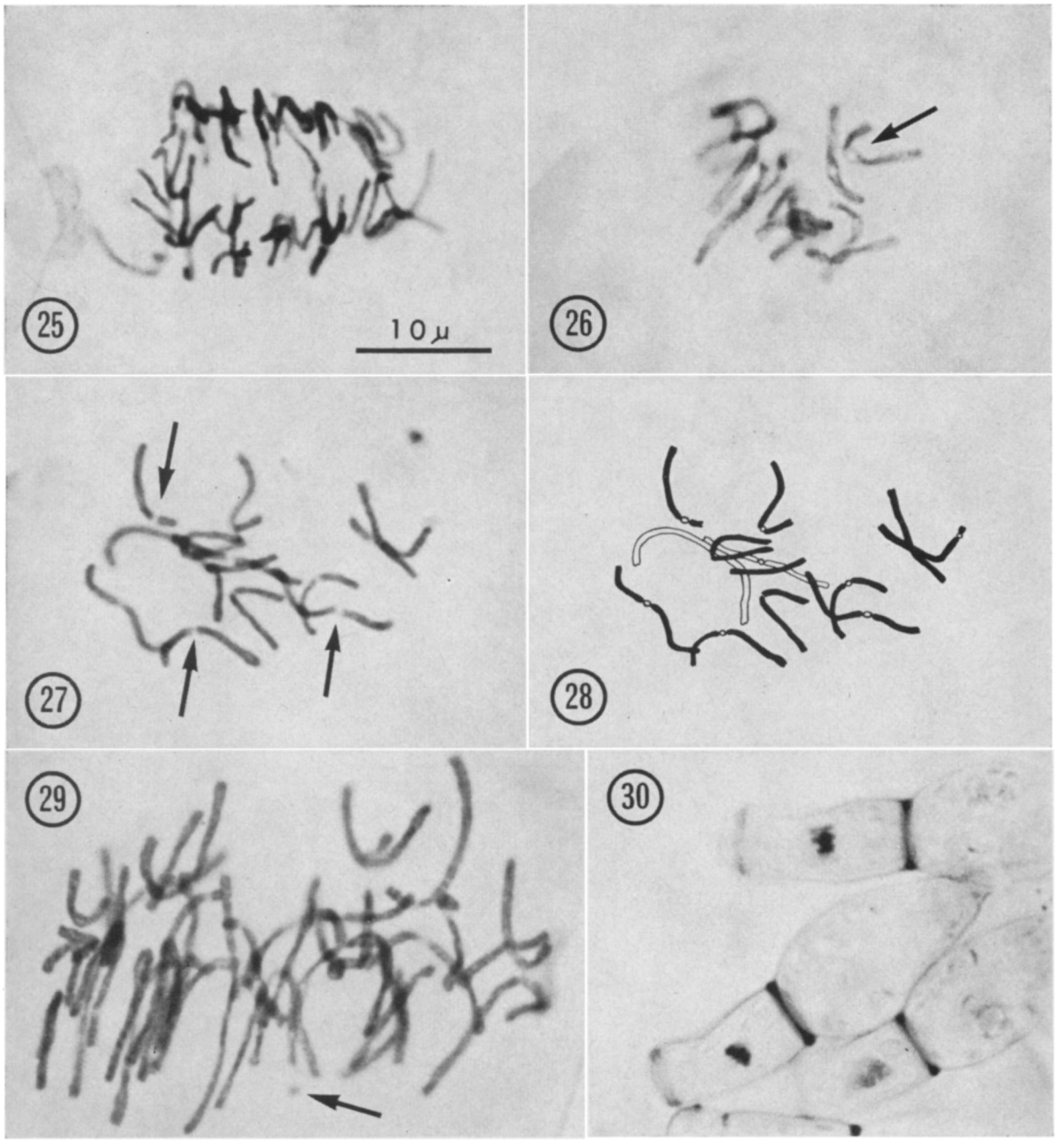


Fig. 25–30. Mitotic chromosomes of *Oedogonium*, all × 2000.—Fig. 25. Squash preparation illustrating the two sets of 13 anaphase chromosomes of *Oe. echinospermum* (androsporangial strain).—Fig. 26. Squash preparation of the 13 chromosomes of *Oe. idioandrosporum* (oogonial strain) at the initiation of anaphase. Chromatids are just beginning to separate in the region of the centromere (arrow).—Fig. 27. Squash preparation of the 17 metaphase chromosomes of *Oe. idioandrosporum* (oogonial strain). Centromeres in this preparation appear as unstained bands (arrows).—Fig. 28. Drawing based on photographs and the original preparation illustrated in Fig. 27.—Fig. 29. Early metaphase chromosomes of an unidentified species (strain 13). Compare size of chromosomes with the chromosomes illustrated in Fig. 30. —Fig. 30. Two groups of metaphase chromosomes of a small-celled, unidentified species (strain 15). Compare with Fig. 29.

TABLE 2. *Chromosome numbers in Oedogonium*[a]

	Species	Haploid (*n*) number	Source
	Macrandrous species		
I.	With bisexual filaments:		
	Oe. vaucherii (Le Cl.) Al. Br.; Wittr.	$n = 16$	Henningsen (1963)
	Oe. foveolatum Wittr.	$n = 16$	Hoffman
	Oe. geniculatum Hirn	$n = 32$[b]	Hoffman
	Oe. terrestris Randhawa	$n = 17$	Chowdary (1963)
II.	With unisexual filaments:		
	Oe. angustistomum Hoffman (male and female strains)	$n = 17$	Hoffman
	Oe. calliandrum Hoffman (male and female strains)	$n = 17$	Hoffman
	Oe. capillare (L.) Kuetz.	$n = 16$	Henningsen (1963)
	Oe. capilliforme Kuetz.; Wittr. (male and female strains)	$n = 17$	Hoffman
	Oe. cardiacum (Hass.) Wittr. (male and female strains)	$n = 19$	Howard and Horsley (1958)[c]; Hasitschka-Jenschke (1960); Sinha (1963); Hoffman
	Oe. grande Kuetz.	$n = 13$	Ohashi (1930)
	Oe. pachyandrium Wittr.	$n = 15$	Kretschmer (1930)
		$n = 17$[d]	Tschermak (1943a)
	Oe. plagiostomum Wittr.	$n = 16$	Henningsen (1963)
	Oe. princeps (Hass.) Wittr. (male and female strains)	$n = 17$	Hoffman
	Oe. pringsheimii Cramer; Wittr.	$n = 18$	Henningsen (1963)
	Nannandrous species		
I.	Gynandrosporous:		
	Oe. ringens Hoffman	$n = 17$	Hoffman
	Oe. setigerum Hoffman	$n = 16$ (17?)[e]	Hoffman
	Oedogonium sp. (strain 13)	$n = 38 \pm 1$	Hoffman
II.	Idioandrosporous:		
	Oe. cyathigerum Wittr.	$n = 19$	van Wisselingh (1908)
	Oe. echinospermum Al. Br. (oogonial and androsporangial strains)	$n = 13$	Hoffman
	Oe. idioandrosporum (Nordst. & Wittr.) Tiff. (oogonial and androsporangial strains)	$n = 13$	Hoffman
	Oe. pluviale Nordst.	$n = 19$	Tschermak (1943a)
	Oe. spectabile Hirn	$n = 16$	Henningsen (1963)
	Unidentified species—No indication of sexual type		
	1 species	$n = 19$	van Wisselingh (1921)
	1 species[f]	$n = 9$	Tschermak (1943a)
	5 species[g]	$n = 13$	Tschermak (1943a)
	3 species	$n = 17$	Tschermak (1943a)
	3 species	$n = 18$	Tschermak (1943a)
	1 species	$n = 19$	Tschermak (1943a)
	1 species	$n =$ ca. 41	Sinha (1963)

[a] All of the counts are based on mitotic material.

[b] Incorrectly given as $n = 33$ in Hoffman (1961).

[c] The chromosome number that was given for *Oedogonium cardiacum* in this paper was apparently derived from the count given by Sinha in his thesis. A later publication by Howard and Horsley (1960) lists the haploid chromosome number for this species as 18.

[d] Tschermak, studying Kretschmer's original preparations, determined a count of $n = 17$ for *Oe. pachyandrium*.

[e] One of the chromosomes in this species appears to be characterized by a relatively long, faintly staining region. Thus, it may readily be mistaken for two chromosomes.

[f] In Lacerda's (1946) reference to Tschermak's work, three species of *Oedogonium* were erroneously reported to have a chromosome number of 9.

[g] A discrepancy appears in Tschermak's (1943a) paper. On page 504 she reports four species studied with a chromosome number of 13, while in the summary (p. 517) five species are listed with 13 chromosomes. The latter figure is assumed to be correct based on the total number of species she reported to have studied. Of the 15 species she studied, 13 were unidentified as indicated here.

The same was true for three heterothallic species studied by Henningsen (1963). Furthermore, no differences could be detected in the chromosome number and morphology of complementary androsporic and female strains of the two idioandrosporous species studied.

DISCUSSION—In the present investigation two species had chromosome numbers not previously reported for the genus. These were *Oe. geniculatum* with 32 chromosomes and an unidentified species (strain 13) with 38 ± 1 chromosomes. The known range of chromosome numbers in *Oedogonium* (Table 2) is from $n=9$ to $n=$ ca. 41. As relatively few species have been examined cytologically, it is likely that the range will be extended further as more work is completed. In this connection it would be especially interesting to study the extremes of small- and large-celled species, since chromosome number and cell size are generally correlated

Tschermak (1943a) postulated $n=9$ as the basic chromosome number in *Oedogonium* and considered that species with 18 chromosomes were derived from the diploid condition. Species with 17 or 19 chromosomes she considered to be aneuploids of the diploid condition. Accordingly, chromosome numbers such as 13, 16, and 32 would imply even greater degrees of aneuploidy. It would appear that the main support for Tschermak's speculation was that the lowest chromosome number known for *Oedogonium* is $n=9$. This count has been reported on only one occasion and was made for a small-celled species with cells about twice the diameter of the author's unidentified species, strain 15 (Fig. 30).

When high chromosome numbers are encountered, there is always the possibility that they represent naturally occurring polyploidy. Cultural studies have shown that diploid strains of *Oedogonium* may arise in several different ways—as a result of atypical oospore germination (Mainx, 1931; Hoffman, 1965) or, perhaps more commonly, by somatic doubling of chromosome number. In either case the resulting diploid strains seem to be quite stable in culture. If these diploid strains could successfully compete with their haploid counterparts in nature, they might represent an important source for the derivation of new species. Tschermak (1943b) has reported the collection of diploid strains of *Oedogonium* from nature which appeared identical to diploid strains which she induced in culture by colchicine treatment. Spontaneous occurrence of polyploids in culture has been noted for a number of algae and is discussed in a paper by Brandham (1965). Brandham also suggests the high probability that diploidy occurs in nature for some of the desmids.

Some variability in the chromosome morphology of a single *Oedogonium* species may occur. Tschermak (1943a) indicated that, for a given species, differences in external growth conditions may result in variability of chromosome size, stainability, and the presence or absence of satellites. She found that chromosomes from old cultures generally stained less satisfactorily and appeared smaller. Furthermore, in one species a distinct satellite was lacking in material from an old culture, but it was clearly present in young culture material. In the present study no such variation of chromosome morphology was observed. However, the material studied was always from young, actively growing cultures, and no comparisons were made with material from aged cultures.

On the basis of chromosome information presently at hand (Table 2), no trends are evident which could be used to help establish taxonomic groups within the genus *Oedogonium*. It is readily apparent, however, that there is great diversity in the genus with regard to chromosome number, size, and morphology.

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COMPARATIVE MORPHOGENESIS OF THE DIMORPHIC LEAVES OF CYAMOPSIS TETRAGONOLOBA[1]

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ABSTRACT

Cyamopsis tetragonoloba, guar, produces two leaf forms: a simple leaf and a trifoliate leaf. The steps in the development of each of these forms have been investigated in an attempt to determine the precise point at which the leaf primordium becomes destined to produce one or the other characteristic leaf shapes. Up to 140 μ in length the leaf primordia are morphologically indistinguishable. If a simple leaf is to be formed the marginal meristem remains continuous and initiates only lamina. If a trifoliate leaf is to be formed the continuity of the marginal meristem is interrupted by a group of "pocketal" cells dividing it into an upper lamina meristem and a basal leaflet buttress.

AN ANGIOSPERM leaf originates as a mound of embryonic cells on the flank of the apical meristem. Eventual form and ultimate size at maturity vary in relation to the direction and amount of growth following initiation.

Primordial development of a simple leaf is similar for many species. The laminae are produced by two marginal meristems formed on the adaxial margins of the primordial axis, whereas compound leaves are formed by various developmental patterns. In *Clematis* the petiole and rachis are formed by apical and intercalary growth of the axis followed by initiation of leaflet primordia on the flanks of the rachis (Tepfer, 1960). A similar pattern is characteristic of *Carya buckleyi*, in which lateral leaflet primordia arise independently on the axis in an acropetal sequence (Foster, 1935). In contrast, an aquatic *Ranunculus* species produces tripartite blades because its primordia branch early in development (Bostrack and Millington, 1962).

Cyamopsis tetragonoloba (L.) Taubert, a legume commonly called guar, has two leaf forms: a simple leaf and a trifoliate leaf. Environmental conditions, such as temperature and photoperiod, can determine when the production of simple leaves will be terminated and followed by subsequent initiation of trifoliate leaves (Fig. 1, 2). The destiny of a primordium is not determined at the time of its initiation. At some later stage in its development environmental factors can influence the nature of its growth and control the ultimate leaf form produced. Since these factors can be controlled, a mechanism is afforded for investigating the ontogenetic basis for the ultimate leaf form. The objective of this study was to determine the cells which are responding to the environmental signals

MATERIALS AND METHODS—The plant used in this study was *Cyamopsis tetragonoloba* 'Texsel.' The seeds were obtained from the 1962 and 1963 Guar Research Plot at the Purdue University Agricultural Experiment Station, Lafayette, Indiana.

Fresh material for comparing the ontogeny of the two leaf forms was collected and immediately killed and fixed in formalin-aceto-alcohol mixture (Johansen, 1940). Air was removed from the material by subjecting it to reduced pressure for several hours. The specimens were usually placed under vacuum again for a short time before dehydration. Dehydration in preparation for paraffin infiltration was by the tertiary-butyl-alcohol method (Johansen, 1940).

The specimens were infiltrated with paraffin;

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